constant dielectric constants 75.25, 66.57 and 54.29 at all three temperatures.

The equation proposed by Amis and Jaffe accounted for the ionic strength data, but revealed a dielectric constant effect which, while in the right direction, was magnified by an order of magnitude. Coulombic energy calculations confirmed the enhanced dielectric constant effect.

These enhanced dielectric constant effects upon the rate in acetone–water media were less by an order of magnitude than has been observed when ethyl alcohol–water was used as a solvent. Thus part of the enhancement is solvent dependent.}

**Chromatographic Separation of Sugars on Charcoal**

**BY ROY L. WHISTLER AND DONALD F. DURSO**

Charcoal has been used industrially for the purification of sugars for many years. In 1932, Hyashi reported that charcoal, stirred in an aqueous acetone–acetic acid solution of glucose and sucrose, completely adsorbed the sucrose but left the glucose in solution. Tiselius proposed that charcoal might be employed in chromatographic fashion for the separation of glucose and lactose. Later, he employed charcoal and 0.5% aqueous phenol solution to effect the separation of glucose from sucrose, and sucrose from maltose by the displacement development technique in which the components are added to the column and then are washed in succession into the effluent without an interval wherein only pure developer is obtained. It was also demonstrated that a mixture of glucose, sucrose, raffinose and stachyose could be separated, using 0.5% aqueous ephedrin as the displacing agent. The method of Tiselius has been used by Claesson to resolve a mixture of glucose, sucrose, raffinose with 4% aqueous phenol; and by Montgomery, Weakley and Hibbert to isolate 6-(α-D-glucopyranosyl)-D-glucose from an enzymic hydrolyzate of starch.

The Tiselius technique has been extended and modified in this Laboratory for use in the fractionation of acid hydrolyzates of guaran and xylan. These separations will be reported later. However, it may be of value to present here the pertinent facts regarding aqueous ethanol as the displacing agent, because its use leads to improved workability of the charcoal technique for the separation of sugars. Preliminary orientation experiments with known sugars indicated that the desorption characteristics of monos-, disaccharides and trisaccharides are so different that they might be easily separated as sugar classes. The effectiveness of the separation is not affected by small variations in the composition of the developer, by the degree of dilution of the sugar solution, or by the presence of inorganic salts. An added advantage is that a mixture of substantially large quantities of sugars can be separated by the use of a single small column.

**Experimental**

**General Procedure.**—The adsorbent used was a mixture of equal parts by weight of Darco G-60 and Celite which had been washed with water and dried. This material was packed into a glass chromatographic tube 250 mm. × 24 mm. diameter, giving a column 170 mm. in length. Before the addition of the sugar solution, the column was wet with 150 ml. of water. In studying the desorption characteristics of the individual sugars, 1 g. in the form of a 10% solution was used, although later work showed that more dilute solutions could be employed with no change in the results.

To effect the displacement of the adsorbed sugars, water and more powerful developers were used in succession. The effluent was collected in 100 ml. fractions. The course of the desorption process was followed polarimetrically, using a 2-dm. tube, to indicate the complete removal of each component before addition of the succeeding developer.

**Desorption of Monosaccharides.**—The method given above was used to study the desorption characteristics of glucose, galactose, mannose, xylose, arabinose, fructose and rhamnose. All of the monosaccharides are displaced at approximately the same rate by water. In all cases, complete removal of the sugar was effected by washing the column with 800 ml. of water. The rates of desorption of four of these sugars are shown in Fig. 1.

Methanol, ethanol, acetic acid and acetone, each in concentrations of 15, 30 and 70% in water, were used to study the effect of other agents upon the ease of displacement of glucose, galactose and mannose. Two hundred ml. of each of these developers, regardless of nature or concentration, caused complete removal of sugar. The increased rates of desorption of glucose with two of these agents are shown in Fig. 1.

**Desorption of Disaccharides.**—The displacement of maltose by various agents was studied to obtain representative data regarding this class of sugars. Maltose was not removed by 2% of water. It was completely desorbed by 900 ml. of 5% ethanol, 400 ml. of 15% ethanol, 800 ml. of 8% citric acid and 1.7 l. of 0.2% phenol. 1.7 liters of 3% hydrochloric acid and 1.1 l. of 7.8% hydrochloric acid gave only 80% recovery of maltose. With 5% ethanol, the displacement of melibiose, lactose, sucrose and

(1) Journal Paper No. 404 of the Purdue University Agricultural Experiment Station, Lafayette, Indiana.

(2) F. Hyashi, J. Biochem. (Japan), 16, 1 (1933); C. A., 27, 8 (1933).


(4) A. Tiselius, Kolloid Z., 105, 101 (1943).

(5) A. Tiselius and L. Hab, ibid., 105, 177 (1943).


(8) A product of Darco Corp., New York, N. Y.

(9) No. 555, a product of Johns-Manville Co., New York, N. Y.
Desorption of Monosaccharides.—Trehalose was effected by 1 l. of developer. The rates of desorption of these disaccharides with 5% ethanol are shown in Fig. 1.

Desorption of Disaccharides.—Trehalose was effected by 1 l. of developer. The rates of desorption of these disaccharides with 5% ethanol are shown in Fig. 2.

Desorption of Trisaccharides.—Raffinose was not removed from a column by the use of water or 5% ethanol. It was slowly removed by 10% ethanol (1.6 l.), but rapidly recovered with 15% ethanol (500 ml.). The rates of desorption of raffinose and melezitose are shown in Fig. 3.

Desorption of Higher Molecular Weight Material.—α-Schardinger dextrin required 30% ethanol for successful displacement. It was not removed by water, 5% ethanol or 15% ethanol.

Separation of Mixtures and Isolation of Components.—A mixture of 1.0 g. each of glucose, maltose and raffinose in the form of a 10% solution was adsorbed on a charcoal column, prepared as previously described, and resolved by the successive use of 800 ml. of water, 1.5 l. of 5% ethanol and 700 ml. of 15% ethanol for displacement of glucose, maltose and raffinose, respectively. Each of these sugars was obtained in crystalline form. One recrystallization served to purify the material sufficiently to give the accepted values of melting point and rotation. In Table I are summarized the results of this work, showing the close agreement between the amount of sugar which was obtained by crystallization, and the recovery as calculated from polarimetric data. The specific rotation of the sugar in the developer was used for this calculation.

### Table I

<table>
<thead>
<tr>
<th>Sugar</th>
<th>Indicated by polarimeter</th>
<th>Actual</th>
</tr>
</thead>
<tbody>
<tr>
<td>Glucose</td>
<td>99</td>
<td>90</td>
</tr>
<tr>
<td>Maltose</td>
<td>95</td>
<td>87</td>
</tr>
<tr>
<td>Raffinose</td>
<td>99</td>
<td>90</td>
</tr>
</tbody>
</table>

In a similar manner, the following mixtures were separated by the above procedure: maltose and raffinose; sucrose, melibiose and raffinose; and glucose, melibiose and raffinose. These mixtures were in the form of 10% solutions containing 1.0 g. of each sugar. The procedure was also successfully applied to the separation of a mixture...
of 5.0 g. of glucose, 1.0 g. of melibiose and 1.0 g. of raffinose dissolved in 2 l. of water.

Separation of Mixtures in the Presence of Salts.—A mixture composed of 7.0 g. of xylose, 0.6 g. of maltose, 0.4 g. of raffinose, 4.0 g. of sodium chloride and 0.4 g. of sodium bicarbonate was dissolved in water and made up to 1 l. Separation of this mixture was accomplished successfully in the above manner. The salts were obtained in the water effluent. Polarimetric data indicated that the separation was complete.

Other salt-containing mixtures which were separated in the same manner were solutions composed as follows: (1) 5.0 g. glucose, 1.0 g. melibiose, 1.0 g. raffinose and 20.0 g. sodium chloride in 1 l. of water; (2) 10.0 g. glucose, 1.0 g. maltose, 0.5 g. raffinose, 30.0 g. sodium chloride and 100 g. sodium bicarbonate in 1.5 l.; (3) 10.0 g. glucose, 1.0 g. maltose, 0.5 g. raffinose, 40.0 g. sodium chloride and 1.0 g. sodium bicarbonate in 1 l.; and (4) 5.0 g. glucose, 1.0 g. maltose, 1.0 g. raffinose and 50.0 g. sodium acetate in 1 l.

Separation of a Mixture of Cellobiose and Raffinose.—Determination of the desorption characteristics of cellobiose showed that this disaccharide was only slowly displaced by 5% ethanol but was rapidly removed by 7.5% ethanol. A mixture of 1.0 g. each of cellobiose and raffinose was separated by washing the column with 7.5% ethanol, of which 1.4 liters were required. However, 300 ml. after the removal of cellobiose, the raffinose began to appear in the effluent. At this point the addition of 15% ethanol was begun and continued until the recovery of raffinose was complete.

Location of Zones on Column.—In order to ascertain the manner in which sugars are adsorbed on charcoal columns, two experiments were performed. In the first, a mixture of 1.0 g. each of glucose and maltose was adsorbed on a 180 mm. X 34 mm. diameter column, which was then washed with water until free of glucose. At this point, the addition of water was discontinued. The column was extruded and cut into 20-mm. sections. Each of these sections was treated with 5% ethanol for removal of maltose, and the optical activity of the filtrate was determined. The polarimetric data indicated that all of the maltose was adsorbed on the upper 100 mm. of charcoal, as shown in Fig. 4A.

In a similar experiment, a mixture of 1.0 g. each of maltose and raffinose was employed. After treatment with 5% ethanol for partial removal of the maltose, the column was extruded and sectioned. Each 20-mm. section was treated first with 5% ethanol for complete removal of any remaining maltose, and then with 15% ethanol until all the raffinose was recovered. As shown in Figure 4B, the raffinose was tightly adsorbed at the top, while the maltose was located at the bottom of the column.

Loading Capacity of Column.—Twenty-five grams of lactose in the form of a 10% solution was added to a 170 X 34 mm. column which was then washed with water until the effluent showed no optical activity. It was found that a large amount of the disaccharide was not adsorbed. On subsequent desorption with 5% ethanol, 2 g. of lactose was recovered, indicating that this amount of sugar represents the loading capacity of the column used.

Fig. 4.—Cross section of charcoal columns showing the manner in which sugars are adsorbed: A, location of maltose zone after treatment of the column to remove all monosaccharides; and B, positions of raffinose and maltose zones after partial removal of the maltose.

Acknowledgments.—The authors wish to express their appreciation to Mrs. Iva M. Buschman and Mrs. Carolyn Johnson for their assistance with the experimental work.

Summary

1. A modification of the Tiselius technique has been developed whereby a mixture of mono-, di- and trisaccharides can be resolved by chromatographic adsorption on charcoal and displacement by water, 5% ethanol and 15% ethanol in succession. The course of the desorption process is followed polarimetrically.

2. Neither the adsorption nor the desorption of sugars is affected by the degree of dilution of the sugar mixture or by the presence of salts such as sodium chloride, sodium bicarbonate and sodium acetate in various concentrations.

3. The use of other developers, such as methanol, ethanol, acetic acid and acetone for monosaccharides, and phenol, citric acid and hydrochloric acid for disaccharides has been investigated.

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